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(54) Title: THERAPEUTIC USES OF THIOL-OXIDIZING AGENTS AND SULFHYDRYL-ALKYLATING AGENTS

(57) Abstract

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The present invention relates to the therapeutic uses of thiol-oxidizing agents and of sulfhydryl-alkylating agents. The sulfhydryl-alkylating agents are maleimide and its derivatives. Therapeutic compositions comprising such agents are also provided. In a specific embodiment, the therapeutic agent is a thiol-oxidizing agent such as a diazene dicarbonyl compound. In another embodiment, the therapeutic agent is maleimide or a derivative thereof. In yet another embodiment, the invention provides methods of treating cystic fibrosis, by administering a therapeutic agent of the invention.

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THERAPEUTIC USES OF THIOL-OXIDIZING AGENTS AND SULFHYDRYL-ALKYLATING AGENTS

1. INTRODUCTION

The present invention relates to thiol-oxidizing agents and sulfhydryl-alkylating agents, and use of such agents for treatment of diseases and disorders, particularly those involving a defective cell membrane, lysosomal, or secretory protein.

2. BACKGROUND OF THE INVENTION

The synthesis and maturation of secretory, lysosomal, and membrane proteins in vertebrate cells involves the participation of various subcellular organelles. After synthesis in the rough endoplasmic reticulum, the protein is moved to the Golgi complex, and then sorted to lysosomes or the plasma membrane or secretory vesicles.

Ribosomes are complexes that carry out protein synthesis within the cell by reading the three letter genetic code (codon) of each messenger RNA. The endoplasmic reticulum (ER) is an interconnected series of flattened, generally layered, sacs within the cell. Ribosomes that are synthesizing secretory and integral membrane (ER, Golgi, and plasma membrane) proteins are tightly bound to the membrane of the ER (which is termed the rough ER with such bound ribosomes). Secretory proteins are transported across the ER membrane into the lumen, or cisterna, of the ER during synthesis; membrane proteins become inserted into the ER membrane during synthesis. A signal sequence, characteristically near the N-terminus of the newly synthesized protein and consisting of one or more positively charged amino acids followed by 6-12 continuous hydrophobic residues, directs a protein to

the ER, and inserts itself into the ER membrane, with the aid of the signal recognition particle. The signal sequence is cleaved off by signal peptidase, localized in the lumen of the ER. Other topogenic sequences within membrane proteins, e.g., stop-transfer membrane anchor sequences, function to orient the protein within the membrane. The protein traverses the ER membrane in an unfolded state. After insertion into or traversal of the ER membrane, the newly synthesized proteins can undergo additional maturation modifications in the ER lumen, including formation of disulfide bonds and proper folding of the protein, formation into oligomers, and addition and modification of carbohydrates. Disulfide bonding stabilizes the tertiary structure of proteins, and is important for proper maturation and activity of the protein. Formation of multi-chain oligomeric proteins from their subunit constituents also occurs in the ER. Polypeptides that are misfolded are prevented from moving out of the ER and proceeding along their normal maturation pathway; such proteins either accumulate or are degraded in the ER via an active degradative pathway (Stafford and Bonifacino, 1991, J. Cell Biol. 115(5):1225-1236; Klausner and Sitia, 1990, Cell 62:611-614; Bonifacino and Lippincott-Schwartz, 1991, Curr. Opin. Cell Biol. 3:592-600).

In eukaryotes, glycosylation of proteins can be classified as O-linked (linked to the hydroxyl group oxygen of serine, threonine, and in collagen, hydroxylysine) or N-linked (linked to the amide nitrogen of asparagine). Glycosyltransferases are enzymes that catalyze the transfer of sugar to newly synthesized proteins; a different type of glycosyltransferase catalyzes the addition of specific sugars. All known glycosyltransferases are integral

membrane proteins with their active sites within the lumen of the ER or Golgi, where sugar transfer thus occurs.

All N-linked oligosaccharides are synthesized from a common precursor in the ER. In the lumen of the ER, the complete branched oligosaccharide, consisting of three glucose, nine mannose, and two N-acetylglucosamine molecules, is transferred by the enzyme oligosaccharyltransferase from oligosaccharylpyrophosphoryldolichol to an asparagine residue in an -Asn-X-Ser/Thr- acceptor site (where X is any amino acid except proline) on the nascent protein (Czichi et al., 1977, J. Biol. Chem. 252:7901-7904; Hart et al., 1979, J. Biol. Chem. 254:9747-9753). Oligosaccharyltransferase is a luminally oriented integral membrane protein of the ER, and the glycosylated protein formed by transfer of the oligosaccharide is sequestered within the endoplasmic reticulum (Hanover and Lennarz, 1980, J. Biol. Chem. 255:3600-3604). An in vitro study has shown that amino-terminal derivatives of Asn-Leu-Thr can act as substrates for oligosaccharyltransferase, while asparagine derivatives of the tripeptide were inactive as substrates or inhibitors of the enzyme (Welply et al., 1983, J. Biol. Chem. 258:11856-11863). A study has suggested that transport from the ER to the cell surface is an unselective process, by comparing the rate of transport of exported proteins with that of an intracellular bulk phase marker; the bulk phase marker used was a tripeptide derivative containing the Asn-X-Ser/Thr acceptor site for glycosylation (Wieland et al., 1987, Cell 50:289-300).

Immediately after transfer of the oligosaccharide to the protein, catalyzed by oligosaccharyltransferase, certain sugar residues are

removed by different enzymes. Further processing of the N-linked oligosaccharide, to the high-mannose or complex form, is completed in the Golgi vesicles. The glycoprotein is transported via transport vesicles from the cis Golgi to the trans Golgi to the trans Golgi reticulum, from where it is sorted to lysosomes or to transport vesicles, or secretory vesicles which eventually fuse with the plasma membrane.

For a general discussion of the foregoing, see Darnell et al., 1990, Molecular Cell Biology, 2d Ed., W.H. Freeman & Co., New York, pp. 639-680; Pfeffer and Rothman, 1987, Ann. Rev. Biochem. 56:829-852.

3. SUMMARY OF THE INVENTION

The present invention relates to the therapeutic uses of thiol-oxidizing agents, and of sulfhydryl-alkylating agents such as maleimide and its derivatives. Therapeutic compositions comprising such agents are also provided. In a specific embodiment, the therapeutic agent is a thiol-oxidizing agent such as a diazene dicarbonyl compound. In another embodiment, the therapeutic agent is maleimide or a derivative thereof. In yet another embodiment, the invention provides methods of treating cystic fibrosis, by administering an effective amount of a therapeutic agent of the invention.

4. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to thiol-oxidizing agents and to sulfhydryl-alkylating agents, and therapeutic uses of the foregoing. Therapeutic methods and compositions are provided. The therapeutic agents of the invention are biocompatible (nontoxic and not highly immunogenic), and permeable

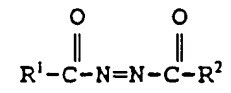
to cell membranes. In a preferred aspect, the therapeutic agent is used in the treatment of a disease or disorder involving a defective cell membrane (plasma, ER, or Golgi), lysosomal, or secretory protein. In a specific embodiment, the therapeutic agent is an agent that prevents the abnormal misfolding, assembly or increased levels of degradation in the ER lumen of a defective lysosomal or secretory or cell membrane (e.g., plasma, Golgi, ER) protein associated with a disease or disorder, thus allowing the protein to proceed along its normal maturation pathway to secretion or to the plasma membrane or a lysosome.

The invention is further detailed in the subsections below.

4.1 THE THIOL-OXIDIZING AGENTS OF THE INVENTION

The thiol-oxidizing agents of the invention are mild oxidants that are cell membrane-permeable. In a preferred aspect, the oxidizing agent inhibits degradation in the ER of a genetically defective protein.

As an example, a thiol-oxidizing agent for use as a therapeutic agent of the invention has the following structure:

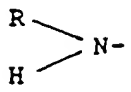


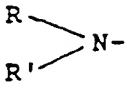
(I)

Compound (I) is a diazene dicarbonyl compound, in which R^1 and R^2 are each independently:

1. straight chain or branched, substituted or unsubstituted, alkyl, aryl, or aralkyl;

2. mono- or di-substituted amino, e.g., the following:





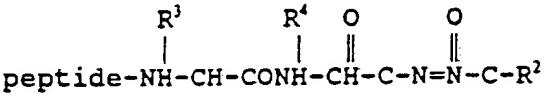
in which R and R' are each independently an alkyl, aryl, or aralkyl group;

3. alkoxy, aryloxy, aralkoxy, e.g., R-O- , in which R is an alkyl, aryl or aralkyl group.

In addition, in compound I,

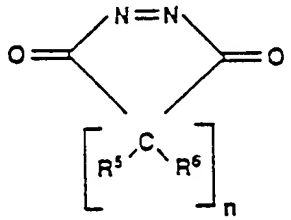
and/or

can be the derivatized C-terminus of a (preferably N-protected) peptide or an amino acid. For example, the oxidizing agent can have the structure



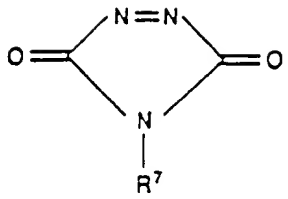
in which R^3 and R^4 are each a side chain of an amino acid. Preferably, in order to maintain cell membrane permeability, the peptide is not larger than a tripeptide.

In compound I, R^1 and R^2 together can also form a ring structure, e.g., compound (II) or (III):



(II)

in which n is an integer of 1 or more; R^5 and R^6 are each independently H, an alkyl, aryl, aralkyl, or the like; where if n > 1, each R^5 and R^6 can be the same or different.

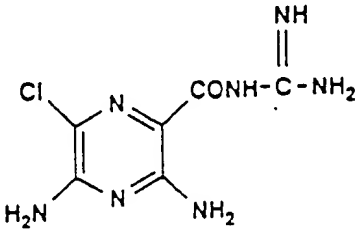


(III)

in which R^7 is H, an alkyl, aryl, or aralkyl or the like.

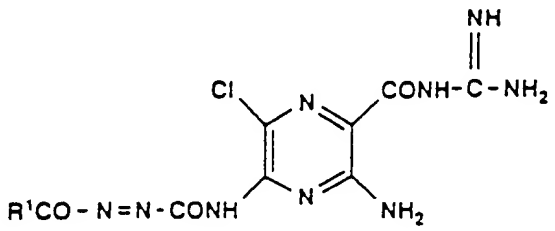
In a preferred embodiment, the oxidizing agent is diazene dicarboxylic acid bis(N,N-dimethylamide) [diamide; $(CH_3)_2NCON=NCON(CH_3)_2$; Kosower et al, 1969, Biochem. Biophys. Res. Comm. 37(4):593-596].

In a specific aspect, the therapeutic agent is a covalent combination or conjugate of a second therapeutic agent with a diazene dicarbonyl compound (I). For example, in the treatment of cystic fibrosis, such a second therapeutic agent can be amiloride (see Nowak, 1991, J. NIH Research 3:40-44). Amiloride has the following structure:

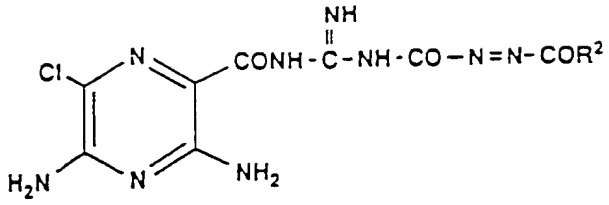


(IV)

A representative active agent comprising a covalent combination of amiloride with compound (I) can have the structure of the compound (V) or (VI), shown below:



(V)

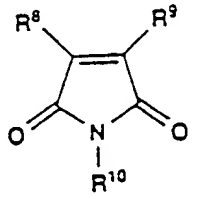


(VI)

4.2 THE SULFHYDRYL-ALKYLATING AGENTS OF THE INVENTION

The sulfhydryl-alkylating agents of the invention are cell membrane-permeable reagents that can alkylate the sulfur atom in the thiol groups of proteins. In a preferred aspect, this alkylation inhibits the degradation in the ER of a genetically defective protein.

The sulfhydryl-alkylating agents are maleimide and its derivatives having the formula (VII):



(VII)

in which R^8, R^9, and R^{10} are each independently H; or a branched or straight chain, substituted or unsubstituted alkyl, aryl, or aralkyl. In a specific embodiment, in which R^8, R^9, and R^{10} are each H, the sulfhydryl-alkylating agent is maleimide. In another

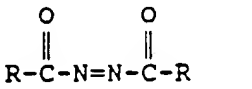
specific aspect, in which R^8 and R^9 are each H, and R^{10} is ethyl, the alkylating agent is N-ethyl-maleimide.

4.3 SYNTHESIS OF THE THERAPEUTIC AGENTS OF THE INVENTION

The therapeutic agents of the invention can be synthesized by methods known in the art, or where available, purchased from a commercial vendor.

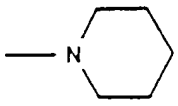
For example, $CH_3CH_2O_2C-N=N-CO_2CH_2CH_3$ is commercially available, or alternatively, can be synthesized as described by Rabjohn (1955, in Organic Syntheses, Collective Vol. 3, Organic Syntheses, Inc., p. 375) or Kauer (1963, in Organic Syntheses, Collective Vol. 4, Organic Syntheses, Inc., p. 411).

Diazene dicarbonyl compounds of formula (VIII)



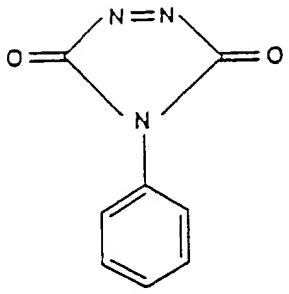
(VIII)

in which R is an alkyl group, can be synthesized as described by Cramer (1957, J. Am. Chem. Soc. 79:6215). Where R is a dimethylamino, or where R is



the synthesis can be carried out as described by Smissman and Makriyannis (1973, J. Org. Chem. 38:1652). The compound of formula (VIII) in which R is NH_2, is commercially available (and used as a maturing and bleaching agent in cereal flour) (Oser et al., 1965, Toxicol. Appl. Pharmacol. 7:445).

The compound 4-phenyl-1,2,4-triazoline-3,5-dione (IX) is commercially available or can be synthesized as described by Cookson et al. (1967, J. Am. Chem. Soc. (C):1905; 1962, Tetrahedron Lett. 615).



(IX)

Many other examples of the therapeutic agents of the invention can be synthesized by the skilled artisan using methods analogous to those described in the art for the foregoing structures.

In a specific embodiment where the therapeutic agent is diazenedicarboxylic acid bis(N,N-dimethylamide), such agent can be easily made as described by Crawford et al. (1963, J. Org. Chem. 28:2419), or purchased from a commercial vendor (e.g., Sigma).

Maleimides can be synthesized by methods known in the art (see, e.g., U.S. Patent No. 4,623,734 granted November 18, 1986 by Masao et al.) or purchased from a commercial vendor.

4.4 IN VITRO ASSAYS

The therapeutic agent of the invention is preferably tested in vitro to ensure that it is permeable to cell membranes. Such assays can be carried out by any method known in the art. In preferred aspects, the assay is carried out by exposing intact cells or rough microsomes or plasma membrane preparations to the agent (which is preferably labeled), and detecting passage through the cell membranes, by methods known in the art (see,

e.g., Stafford and Bonifacino, 1991, J. Cell. Biol. 115(5):1225-1236); Welply et al., 1983, J. Biol. Chem. 258:11856-11863; Wieland et al., 1987, Cell 20:289-300). Rough microsomes are small closed vesicles formed by fragments of the rough ER produced upon homogenization of cells; microsomes have the same orientation (ribosomes on the outside of the vesicles) as that of the ER within the cell (Darnell et al., 1990, Molecular Cell Biology, 2d Ed., W.H. Freeman & Co., New York, p. 646).

4.5 UTILITY OF THE INVENTION

The oxidizing and alkylating agents of the invention can be administered therapeutically, where a therapeutic effect is mediated by the agent upon oxidation or alkylation, as the case may be, of a protein in the body of a subject. In a preferred aspect, the protein is a genetically defective protein, in particular, a lysosomal, secretory, or cell membrane protein.

The therapeutic methods of the invention are carried out by administration to a subject of a therapeutically effective amount of an agent of the invention. The subject is preferably a mammal, including but not limited to animals such as cows, pigs, etc., and is most preferably human.

Methods for prevention of disorders, by administering a therapeutic agent of the invention, are also provided.

In a preferred embodiment, the agent is administered to a patient for treatment of a disorder involving a genetically mutated lysosomal or secretory or plasma, ER, or Golgi membrane protein. Although Applicants do not intend to be limited to any specific mechanism, it is believed that delivery of such an

agent to the ER lumen allows oxidation or alkylation, as the case may be, of cysteine sulfhydryl groups therein, thereby permitting proper folding and cellular targeting of the protein that otherwise would not occur, or preventing its degradation. Diseases or disorders which can be treated in this manner include but are not limited to cystic fibrosis, emphysema, Tay-Sachs, lysosomal storage diseases, insulin receptor deficiency, familial hypercholesterolemia, Hunter's syndrome, and Hurler's syndrome. As discussed infra, cystic fibrosis is associated with a mutation in the transmembrane protein CFTR. The major genetic cause of emphysema and difficulty in breathing is due to a mutation in the secretory protein α,-antiprotease (α,-antitrypsin) (Darnell et al., 1990, Molecular Cell Biology, 2d Ed., W.H. Freeman & Co., New York, p. 660). Tay-Sachs disease is caused by a defect in the lysosomal enzyme beta-N-hexosaminidase A (id., p. 671). Other lysosomal storage diseases are caused by defective lysosomal enzymes. Insulin receptor deficiency results from a mutant (plasma membrane) insulin receptor, while familial hypercholesterolemia results from a mutant LDL (low density lipoprotein) (plasma membrane) receptor. Hunter's syndrome and Hurler's syndrome are caused by genetic defects in the lysosomal enzymes which catabolize sulfated mucopolysaccharides (Darnell et al., supra at p. 671).

In a specific embodiment, the therapeutic agent of the invention is administered to treat cystic fibrosis. Cells from cystic fibrosis patients show a defect in the putative protein product of the cystic fibrosis gene (Rommens et al., 1989, Science 245:1059-1065; Riordan et al., 1989, Science 245:1066-1073; Kerem et al., 1989, Science 245:1073-1080) designated

the CFTR (cystic fibrosis transmembrane conductance regulator; Riordan et al., 1989, Science 245:1066-1073). CFTR is an integral membrane protein that appears to act as a chloride channel (Anderson et al., 1991, Cell 67:775-784; Rich et al., 1990, Nature 347:358-363; Drumm et al., 1990, Cell 62:1277-1233). The present invention provides for treatment of cystic fibrosis by exposure of mutant CFTR in the lumen of the ER, to the oxidizing or alkylating agent that is the therapeutic agent of the invention. Although Applicants do not intend to be limited to a specific mechanism, it is believed that the oxidizing or alkylating agent inhibits degradation and/or promotes the correct folding/assembly in the ER lumen of the mutant CFTR that otherwise would be abnormally processed and never reach the plasma membrane, thus achieving proper processing of CFTR to the cell membrane. The agent is administered so as to allow, or preferably target, delivery to the in vivo cellular location of CFTR, (Crawford et al., 1991, Proc. Natl. Acad. Sci. USA 88:9262-9266), namely epithelial cells, such as those lining sweat ducts, small pancreatic ducts, and intestinal crypts, and in the kidney, and in the lung.

In another embodiment, directed to the treatment of cystic fibrosis, the therapeutic agent is a covalent combination of amiloride and a diazene dicarbonyl compound (I), e.g., diamide. In a most preferred aspect, such an agent is administered in combination with adenosine triphosphate (ATP) and uridine triphosphate (UTP) (see Nowak, 1991, J. NIH Research 3:40-44).

Suitable in vitro and in vivo assays can be used to demonstrate therapeutic utility of the conjugates of the invention. For in vivo testing,

glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The agents of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the oxidizing or alkylating agent of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention.

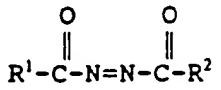
The present invention is not to be limited in scope by the specific embodiments described herein

since such embodiments are intended as but single illustrations of one aspect of the invention and any embodiments which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference herein in their entireties.

WHAT IS CLAIMED IS:

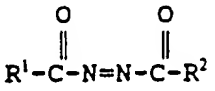
1. A pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (I)



(I)

in which R^1 and R^2 are each independently a straight chain or branched, substituted or unsubstituted, alkyl, aryl, or aralkyl; a mono- or di-substituted amino; or an alkoxy, aryloxy, or aralkoxy; and a pharmaceutically acceptable carrier.

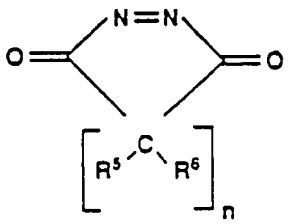
2. A pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (I)



(I)

in which R^1-C(=O)- and R^2-C(=O)- are each independently the derivatized carboxy-terminus of an amino acid or a peptide; and a pharmaceutically acceptable carrier.

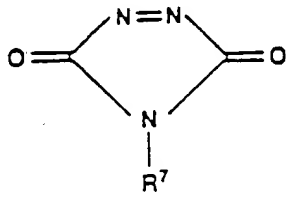
3. A pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (II)



(II)

in which n is an integer of 1 or more; R^5 and R^6 are each independently H, and alkyl, aryl, or aralkyl; where if n is greater than 1, each R^5 can be the same or different, and each R^6 can be the same or different; and a pharmaceutically acceptable carrier.

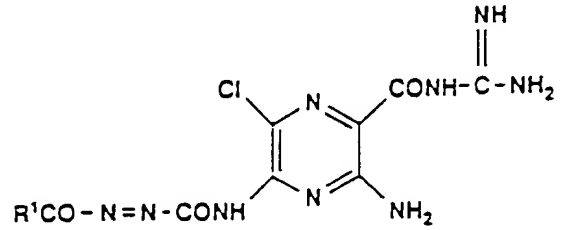
4. A pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (III)



(III)

in which R^7 is an alkyl, aryl or aralkyl; and a pharmaceutically acceptable carrier.

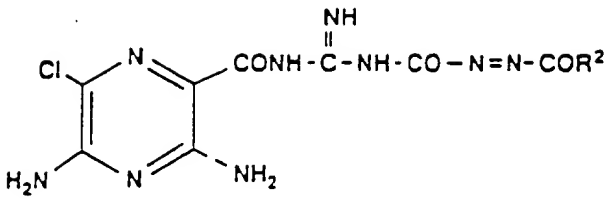
5. A pharmaceutical composition comprising an effective amount of a compound of formula (V)



(V)

in which R^1 is a straight chain or branched alkyl, aryl, or aralkyl; a mono- or di-substituted amino; or an alkoxy, aryloxy, or aralkoxy; and a pharmaceutically acceptable carrier.

6. A pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (VI)

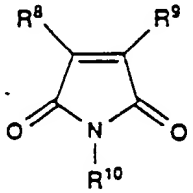


(VI)

in which R^2 is a straight chain or branched alkyl, aryl, or aralkyl; a mono- or di-substituted amino; or an alkoxy, aryloxy, or aralkoxy; and a pharmaceutically acceptable carrier.

7. A pharmaceutical composition comprising a therapeutically effective amount of diamide, of formula $(CH_3)_2NCON=NCON(CH_3)_2$; and a pharmaceutically acceptable carrier.

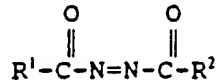
8. A pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (VII)



(VII)

in which R^8, R^9, and R^{10} are each independently H; a straight chain or branched, substituted or unsubstituted, alkyl, aryl, or aralkyl; and a pharmaceutically acceptable carrier.

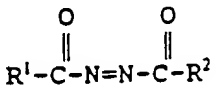
9. A method of treating a disease or disorder in a mammal comprising administering to the mammal a therapeutically effective amount of a compound of formula (I)



(I)

in which R^1 and R^2 are each independently a straight chain or branched, substituted or unsubstituted, alkyl, aryl, or aralkyl; a mono- or di-substituted amino; or an alkoxy, arylcxy, or aralkoxy.

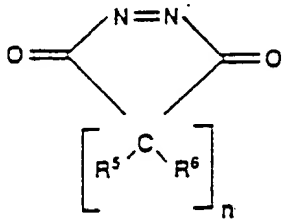
10. A method of treating a disease or disorder in a mammal comprising administering to the mammal a therapeutically effective amount of a compound of formula (I)



(I)

in which R^1-C(=O)- and R^2-C(=O)- are each independently the derivatized carboxy-terminus of an amino acid or a peptide.

11. A method of treating a disease or disorder in a mammal comprising administering to the mammal a therapeutically effective amount of a compound of formula (II)

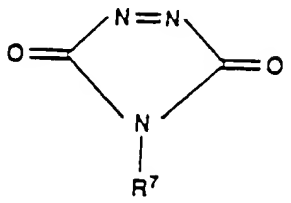


(II)

in which n is an integer of 1 or more; R^5 and R^6 are each independently H, and alkyl, aryl, or aralkyl; where if n is greater than 1, each R^5 can be the same or different, and each R^6 can be the same or different.

12. A method of treating a disease or disorder in a mammal comprising administering to the

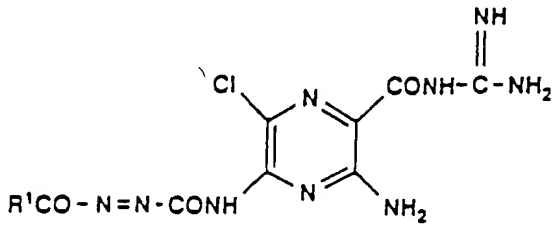
mammal a therapeutically effective amount of a compound of formula (III)



(III)

in which R^7 is an alkyl, aryl or aralkyl.

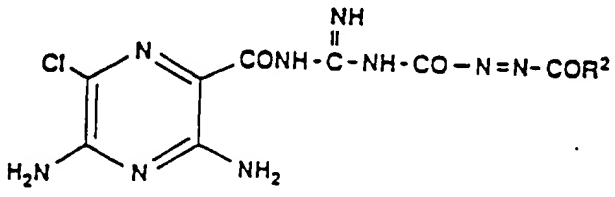
13. A method of treating a disease or disorder in a mammal comprising administering to the mammal a therapeutically effective amount of a compound of formula (V)



(V)

in which R^1 is a straight chain or branched alkyl, aryl, or aralkyl; a mono- or di-substituted amino; or an alkoxy, aryloxy, or aralkoxy.

14. A method of treating a disease or disorder in a mammal comprising administering to the mammal a therapeutically effective amount of a compound of formula (VI)

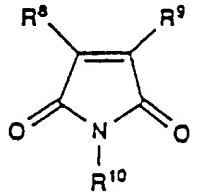


(VI)

in which R^2 is a straight chain or branched alkyl, aryl, or aralkyl; a mono- or di-substituted amino; or an alkoxy, aryloxy, or aralkoxy.

15. A method of treating a disease or disorder in a mammal comprising administering to the mammal a therapeutically effective amount of diamide, of formula $(CH_3)_2NCON=NCON(CH_3)_2$.

16. A method of treating a disease or disorder in a mammal comprising administering to the mammal a therapeutically effective amount of a compound of formula (VII)



(VII)

in which R^8, R^9, and R^{10} are each independently H; a straight chain or branched, substituted or unsubstituted, alkyl, aryl, or aralkyl.

17. The method according to claim 9 in which the disease or disorder involves a genetically defective lysosomal, secretory, or cell membrane protein, and the mammal has or is suspected of having the disease or disorder.

18. The method according to claim 10 in which the disease or disorder involves a genetically defective lysosomal, secretory, or cell membrane protein, and the mammal has or is suspected of having the disease or disorder.

19. The method according to claim 11 in which the disease or disorder involves a genetically defective lysosomal, secretory, or cell membrane protein, and the mammal has or is suspected of having the disease or disorder.

20. The method according to claim 12 in which the disease or disorder involves a genetically defective lysosomal, secretory, or cell membrane protein, and the mammal has or is suspected of having the disease or disorder.

21. The method according to claim 13 in which the disease or disorder involves a genetically defective lysosomal, secretory, or cell membrane protein, and the mammal has or is suspected of having the disease or disorder.

22. The method according to claim 15 in which the disease or disorder involves a genetically defective lysosomal, secretory, or cell membrane protein, and the mammal has or is suspected of having the disease or disorder.

23. The method according to claim 16 in which the disease or disorder involves a genetically defective lysosomal, secretory, or cell membrane protein, and the mammal has or is suspected of having the disease or disorder.

24. The method according to claim 9 in which the disease or disorder is cystic fibrosis, and the mammal has or is suspected of having cystic fibrosis.

25. The method according to claim 10 in which the disease or disorder is cystic fibrosis, and the mammal has or is suspected of having cystic fibrosis.

26. The method according to claim 11 in which the disease or disorder is cystic fibrosis, and the mammal has or is suspected of having cystic fibrosis.

27. The method according to claim 12 in which the disease or disorder is cystic fibrosis, and the mammal has or is suspected of having cystic fibrosis.

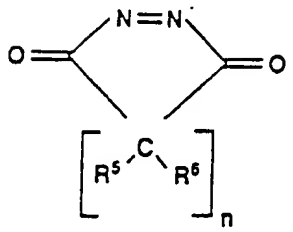
28. The method according to claim 15 in which the disease or disorder is cystic fibrosis, and the mammal has or is suspected of having cystic fibrosis.

29. A compound of formula (I)

(I)

in which  and  are each independently the derivatized carboxy-terminus of an amino acid or a peptide.

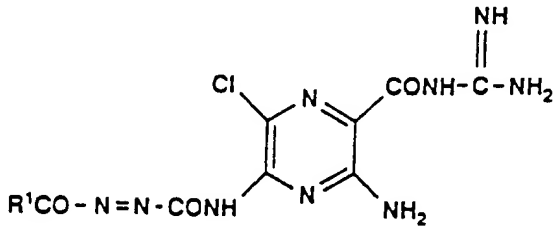
30. A compound of formula (II)



(II)

in which n is an integer of 1 or more; R^5 and R^6 are each independently H, and alkyl, aryl, or aralkyl; where if n is greater than 1, each R^5 can be the same or different, and each R^6 can be the same or different.

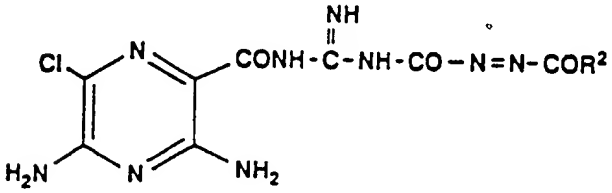
31. A compound of formula (V)



(V)

in which R^1 is a straight chain or branched alkyl, aryl, or aralkyl; a mono- or di-substituted amino; or an alkoxy, aryloxy, or aralkoxy.

32. A compound of formula (VI)



(VI)

in which R^2 is a straight chain or branched alkyl, aryl, or aralkyl; a mono- or di-substituted amino; or an alkoxy, aryloxy, or aralkoxy.

33. The method according to claim 9 in which the mammal is a human.

34. The method according to claim 10 in which the mammal is a human.

35. The method according to claim 11 in which the mammal is a human.

36. The method according to claim 12 in which the mammal is a human.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00045

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 3,017,406 (MEHR) 16 January 1962, See the entire document.	1,2
X	US, A, 3,192,196 (VIDAL ET AL.) 06 June 1965, See the entire document.	1,2
X	US, A, 3,347,845 (SHEPPARD ET AL. I) 17 October 1967, See the entire document.	1,2
X	US, A, 3,366,622 (CHALLINOR ET AL.) 30 January 1968, See the entire document.	1,2
X	US, A, 3,522,233 (SHEPPARD ET AL. II) 28 July 1970, See the entire document.	1,2,7

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be part of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 MARCH 1993	0 3 MAY 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer FLOYD D. HIGEL Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00045

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BER. DEUT. CHEM. GESELL., VOL. 99, 1966, (HANS BOCK ET AL.) "Substituenten-Effekte bei Azodicarbonsäure-Derivaten und ihre Deutung durch Hückel-MO-Rechnungen", pages 2039 to 2051, See entire document.	1,2,7
X	J. ORG. CHEM., VOL. 28, 1963, (ROBERT J. CRAWFORD ET AL.," The Synthesis and Reactions of N,N'-Dicarbalkoxy-N,N'-dialkoxyhydrazines and Some Observations on carbalkoxylium Ions, pages 2419 to 2424, See the entire document.	1,2,7
X	US, A, 3,966,530 (CUTTS ET AL.) 29 June 1976, See the entire document.	4
X	US, A, 4,481,356 (GILBERTSON) 06 November 1984, See the entire document.	4
X	INDIAN J. CHEM., VOL. 14B, 1976, (V.P. ARYA ET AL.), "Synthesis of Novel Heterocycles: Part XXII Syntheses of Novel Heterocycles from 4-Substituted 1,2,4-Triazolidin-3,5-diones", pages 883 to 886. See entire document.	4
X	US, A, 2,205,558 (FLETT ET AL.) 25 June 1940, See entire document.	8,16,23
X	US, A, 3,337,584 (KNOCK) 22 August 1967, See the entire document.	8,16,23
X	US, A, 4,542,225 (BLATTLER ET AL.) 17 September 1985, See entire document.	8,16,23
X	JA, A, 53-98960 (TAKATORI Y) 29 August 1978, See the entire document.	8,16,23
X	CHEMICAL ABSTRACTS, VOL. 77, 1972, NO. 2190a, (EDWARD M. KOSOWEV ET AL.,) "Glutathione, VII. Differentiation among Substrates by the thiol-oxidizing agent, diamide", See the entire document.	9,15,17,22, 24,28,33
X	CHEMICAL ABSTRACTS, VOL. 88, 1978, NO. 696392, (MARLENE M. HOSEY ET AL.), "Inhibition of protein phosphorylation and induction of protein cross-linking in erythrocyte membranes by diamide", See the entire document.	9,15,17,22, 24,28,33
X	CHEMICAL ABSTRACTS, VOL. 94, 1981, NO. 116438u, (JOSEPH KURANTSIN-MILLS ET AL.) "Aggregation of intramembrane particles in erythrocyte membranes treated with diamide", See the entire document.	9,15,17,22, 24,28,33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00045

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, VOL. III, 1989, NO. 128496u, (MICHAEL L. FREEMAN ET AL.), "Modulationm of diamide toxicity in thermotolerant cells by inhibition of protein synthesis", See the entire document.	9,15,17,22, 24,28,33
X	CHEMICAL ABSTRACTS, VOL. 112, 1990, NO. 94687k, (MARGARET A. BAKER ET AL.) "Diamide induced shift in protein and glutathione thiol: disulfide status delays DNA rejoining after x-irradiation of human cancer cells", See the entire document.	9,15,17,22, 24,28,33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00045

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61K 31/40, 31/41, 31/415, 31/665; C07D 207/40, 231/04, 231/08, 231/28, 237/00, 237/02, 249/12, 249/16, 403/00, 487/00; C07C 245/02,245/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/150,247,384,404,425; 534738,751,886; 544/240;548/263.6, 366.4,548